IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

DECLARATION OF VISHWANATH R. IYER, Ph.D. UNDER 37 C.F.R. § 1.132

I, VISHWANATH R. IYER, Ph.D., declare and state as follows:

1. I am an Assistant Professor in the Section of Molecular Genetics and Microbiology, Institute of Cellular and Molecular Biology, University of Texas at Austin, where my laboratory currently studies global transcriptional control in yeast, gene expression programs during human cell proliferation, and genome-wide transcription factor targets in yeast and human. Immediately prior to this position, I spent four years as a postdoctoral fellow in the laboratory of Patrick O. Brown at Stanford University studying the transcriptional programs of yeast and of human cells. My *curriculum vitae* is attached hereto as Exhibit A.

2. Beginning in Dr. Brown's laboratory, where I helped to develop the first whole genome arrays for yeast and early versions of highly representative cDNA arrays for human cells, and continuing to the present day, I have used microarray-based gene expression analysis as a principal approach in much of my research.

3. Representative publications describing this work include:

DeRisi J. *et al.*, "Exploring the metabolic and genetic control of gene expression on a genomic scale," *Science* 278:680-686 (1997);[1]

Marton *et al.*, "Drug target validation and identification of secondary drug target effects using DNA microarrays," Nature Med. 4:1293-1301 (1998);[2]

Iyer *et al.*, "The transcriptional program in the response of human fibroblasts to serum," Science 283:83-87 (1999);[3] and

Ross *et al.*, "Systematic variation in gene expression patterns in human cancer cell lines," *Nature Genetics* 24: 227-235 (2000).[4]

Two of the papers describe our use of microarray-based expression profiling to explore the metabolic reprogramming that occurs during major environmental changes, both in yeast (DeRisi *et al.*, during the shift from fermentation to respiration) and in human cells (Iyer *et al.*, human fibroblasts exposed to serum). One reference describes our use of expression profile analysis in drug target validation and identification of secondary drug effects (Marton *et al.*). And one describes our use of expression profiling as a molecular phenotyping tool to discriminate among human cancer cells (Ross *et al.*).

4. Whether used to elucidate basic physiological responses, to study primary and secondary drug effects, or to discriminate and classify human cancers, expression profiling

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[1] Attached hereto as Exhibit B.

[2] Attached hereto as Exhibit C.

[3] Attached hereto as Exhibit D.

[4] Attached hereto as Exhibit E.

as we have practiced it relies for its power on comparison of ***patterns*** of expression.

5. For example, we have demonstrated that we can use the presence or absence of a characteristic drug "signature" pattern of altered gene expression in drug-treated cells to explore the mechanism of drug action, and to identify secondary effects that can signal potentially deleterious drug side effects. As another example, we have demonstrated that gene expression patterns can be used to classify human tumor cell lines. While it is of course advantageous to know the biological function of the encoded gene products in order to reach a better understanding of the cellular mechanisms underlying these results, these pattern-based analyses do not require knowledge of the biological function of the encoded proteins.

6. The resolution of the patterns used in such comparisons is determined by the number of genes detected: the greater the number of genes detected, the higher the resolution of the pattern. It goes without saying that higher resolution patterns are generally more useful in such comparisons than lower resolution patterns. With such higher resolutions comes a correspondingly higher degree of statistical confidence for distinguishing different patterns, as well as identifying similar ones.

7. Each gene included as a probe on a microarray provides a signal that is specific to the cognate transcript, at least to a first approximation.[5] Each new gene-specific

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[5] In a more nuanced view, it is certainly possible for a probe to signal the presence of a variety of splice variants of a single gene,

(Continued...)

probe added to a microarray thus increases the number of genes detectable by the device, increasing the resolving power of the device. As I note above, higher resolution patterns are generally more useful in comparisons than lower resolution patterns. Accordingly, each new gene probe added to a microarray increases the usefulness of the device in gene expression profiling analyses. This proposition is so well-established as to be virtually an axiom in the art, and has been as long as I have been working in the field, and certainly since the time I embarked on the production of whole genome arrays in early 1996. Simply put, arrays with fewer gene-specific probes are inferior to arrays with more gene-specific probes.

8. For example, our ability to subdivide cancers into discriminable classes by expression profiling is limited by the resolution of the patterns produced. With more genes contributing to the expression patterns, we can potentially draw finer distinctions among the patterns, thus subdividing otherwise indistinguishable cancers into a greater number of classes; the greater the number of classes, the greater the likelihood that the cancers classified together will respond similarly to therapeutic intervention, permitting better individualization of therapy and, we hope, better treatment outcomes.

9. If a gene does not change expression in an experiment, or if a gene is not expressed and produces no

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without discriminating among them, and for a probe to signal the presence of a variety of allelic variants of a single gene, again without discriminating among them.

signal in an experiment, that is not to say that the probe lacks usefulness on the array; it only means that an insufficient number of conditions have been sampled to identify expression changes. In fact, an experiment showing that a gene is not expressed or that its expression level does not change can be equally informative. To provide maximum versatility as a research tool, the microarray should include -- and as a biologist I would want my microarray to include -- each newly identified gene as a probe.

10. I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and may jeopardize the validity of any patent application in which this declaration is filed or any patent that issues thereon.

Vishwanath

VISHWANATH R. IYER, Ph.D.

October 20, 2003

Date

Vishwanath R. Iyer

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Education/Training

Bombay University, Mumbai, India	B.Sc. (1987), Chemistry & Biochemistry
M. S. University of Baroda, Baroda, India	M.Sc. (1989), Biotechnology
Harvard University, Cambridge MA	Ph.D. (1996), Genetics
Stanford University, Stanford CA	Post-doctoral (1996-2000), Genomics

Research Experience

9/00-5/03 Assistant professor, Section of Molecular Genetics and Microbiology, University of Texas, Austin TX

- Global transcriptional control in yeast
- Gene expression programs during human cell proliferation
- Genome-wide transcription factor targets in yeast and human
- Collaborative microarray facility

5/96-8/00 Post-doctoral fellow Stanford University, Stanford CA
(Advisor: Dr. Patrick O. Brown)

- Yeast whole-genome ORF and intergenic microarrays
- Human cDNA microarrays for expression profiling

9/89-4/96 Graduate student Harvard University, Cambridge MA
(Advisor: Dr. Kevin Struhl)

- Yeast transcriptional regulation

Honours and Awards

Government of India Biotechnology Fellowship (1987-1989)
University Grants Commission Junior Research Fellowship (1989)
Stanford University/NHGRI Genome Training Grant (1996)

Invited Conference talks (selected)

Invited Lecturer, NEC-Princeton Lectures in Biophysics
Princeton, NJ (June 1998)
Plenary Session Speaker, HGM '99 (HUGO Human Genome Meeting)
Brisbane, Australia (April 1999)
Invited Speaker, Gordon Research Conference "Human Molecular Genetics"
Newport, RI (August 2001)

Invited Speaker, Nature Genetics "Oncogenomics 2002" Conference
Dublin, Ireland (May 2002)
Invited Speaker, "Pathology Bioinformatics" Symposium, University of Michigan, Ann Arbor, MI (November 2002)
Invited Speaker, "Systems Biology: Genomic Approaches to Transcriptional Regulation" Cold Spring Harbor Laboratory Meeting (March 2003)
Symposium co-Chair and Speaker "Functional Genomics" American Society for Biochemistry and Molecular Biology Meeting, San Diego, CA (April 2003)
Invited Speaker in Functional Genomics (Gene Networks) Symposium, International Congress of Genetics, Melbourne Australia July 6-11 2003
Invited Speaker "BioArrays Europe 2003"
Cambridge, UK (Sep/Oct 2003)

Departmental Seminars

Texas A&M University Genetics and Biochemistry & Biophysics Departments, October 24 2002
New York University School of Medicine, Department of Biochemistry, November 20 2002
UT Southwestern Medical Center, Human Genetics Seminar Series, May 5 2002
UCLA School of Medicine, Department of Human Genetics
June 2 2003
National Human Genome Research Institute
June 12 2003
Sanger Institute of the Wellcome Trust, Hinxton, UK
Sep 2003

Other Professional Activities

Reviewer for *Genome Biology, Genome Research, Nature Genetics, Science* (1998-2003)
Instructor, Cold Spring Harbor Summer Course "Making and using DNA Microarrays" (2000 - 2003)
Member, NIDDK Special Emphasis Review Panel ZDK1 (2001-2002)

Publications

1. Iyer V. & Struhl, K. (1995) Poly(dA:dT), a ubiquitous promoter element that stimulates transcription via its intrinsic DNA structure, *EMBO J.* 14: 2570-2579.

2. Iyer V. & Struhl, K. (1995) Mechanism of differential utilization of the his3 TR and TC TATA elements, *Mol. Cell. Biol.* 15: 7059-7066.

3. Iyer V. & Struhl K. (1996) Absolute mRNA levels and transcription initiation rates in *Saccharomyces cerevisiae. Proc. Natl. Acad. Sci*. (*USA*) 93:5208-5212.

4. DeRisi J. L., Iyer V. R. & Brown P. O. (1997) Exploring the metabolic and genetic control of gene expression on a genomic scale. *Science* 278:680-686

5. Marton M. J., DeRisi J. L., Bennett H. A., Iyer V. R., Meyer M. R., Roberts C. J., Stoughton R., Burchard J., Slade D., Dai H., Bassett D. E. Jr., Hartwell L. H., Brown P. O. & Friend S. H. (1998) Drug target validation and identification of secondary drug target effects using DNA microarrays. *Nature Med.* 4:1293-1301

6. Lutfiyya L. L., Iyer V. R., DeRisi J., DeVit M. J., Brown P. O. & Johnston M. (1998) Characterization of three related glucose repressors and genes they regulate in *Saccharomyces cerevisiae*. *Genetics* 150:1377-1391

7. Spellman P. T., Sherlock G., Zhang M. Q., Iyer V. R., Anders K., Eisen M. B., Brown P. O., Botstein D. & Futcher B. (1998) Comprehensive identification of cell cycle-regulated genes of the yeast *Saccharomyces cerevisiae* by microarray hybridization. *Mol. Biol. Cell* 9:3273-3297

8. Iyer V. R., Eisen M. B., Ross D. T., Schuler G., Moore T., Lee J. C.,F., Trent J. M., Staudt L. M., Hudson Jr. J., Boguski M. S., Lashkari D., Shalon D., Botstein D. & Brown P. O. (1999) The transcriptional program in the response of human fibroblasts to serum. *Science* 283:83-87

9. DeRisi J. L. & Iyer V. R. (1999) Genomics and array technology. *Curr. Opin. Oncol.* 11:76-79

10. Ross D. T., Scherf U., Eisen M. B., Perou C. M., Spellman P., Iyer V. R., Rees C., Jeffrey S. S., Van de Rijn M., Waltham M., Pergamenschikov A., Lee J. C. F., Lashkari D., Shalon D., Myers T. G., Weinstein J. N., Botstein D., & Brown P. O. (2000) Systematic variation in gene expression patterns in human cancer cell lines. *Nature Genetics* 24: 227-235

11. Sudarsanam P., Iyer V. R., Brown P. O. & Winston F. (2000) Whole-genome expression analysis of *snf/swi* mutants of *S. cerevisiae*. *Proc. Natl. Acad. Sci .(USA)* 97: 3364-3369

12. Tran H. G., Steger D. J., Iyer V. R., & Johnson A. D. (2000) The chromo domain protein Chd1p from budding yeast is an ATP-dependent chromatin-modifying factor *EMBO J* 19: 2323-2331

13. Gross C., Kelleher M., Iyer V. R., Brown P. O., & Winge D. R.. (2000) Identification of the copper regulon in *Saccharomyces cerevisiae* by DNA microarrays. *J. Biol. Chem.* 275: 32310-32316

14. Reid J. L., Iyer V. R., Brown P. O. & Struhl K. (2000) Coordinate regulation of yeast ribosomal protein genes is associated with targeted recruitment of Esa1 histone acetylase. *Mol. Cell* 6: 1297–1307

15. Iyer V. R., Horak C., Scafe C. S., Botstein D., Snyder M. & Brown P. O. (2001) Genomic binding sites of the yeast cell-cycle transcription factors SBF and MBF *Nature* 409: 533-538

16. Miki R., Kadota K., Bono H., Mizuno Y., Tomaru Y., Carninci P., Itoh M., Shibata K., Kawai J., Konno H., Watanabe S., Sato K., Tokusumi Y., Kikuchi N., Ishii Y., Hamaguchi Y., Nishizuka I., Goto H., Nitanda H., Satomi S., Yoshiki A., Kusakabe M., DeRisi J.L., Eisen M.B., Iyer V.R., Brown P.O., Muramatsu M., Shimada H., Okazaki Y. & Hayashizaki Y. (2001) Delineating developmental and metabolic pathways in vivo by expression profiling using the RIKEN set of 18,816 full-length enriched mouse cDNA arrays *Proc. Natl. Acad. Sci.* (*USA*) 98: 2199-2204

17. Pollack J. R. & Iyer V.R. (2002) Characterizing the physical genome. *Nature Genetics* 32 suppl: 515-521

18. Iyer V. R. Microarray-based detection of DNA protein interactions: Chromatin Immunoprecipitation on Microarrays, in *DNA Microarrays: A Molecular Cloning Manual* (eds. Bowtell, D. & Sambrook, J.) 453-463 (Cold Spring Harbor Laboratory Press, 2003).
*(*not peer reviewed*)

19. Killion, P., Sherlock G. and Iyer V. R. (2003) The Longhorn Array Database, an open-source implementation of the Stanford Microarray Database *BMC Bioinformatics* 4: 32

20. Hahn J. S., Hu Z., Thiele D. J. & Iyer V. R. Genome-Wide Analysis of the Biology of Stress Responses Through Heat Shock Transcription Factor (submitted to *PNAS*)

21. Kim J. & Iyer V.R. The global role of TBP recruitment to promoters in mediating gene expression profiles (manuscript in preparation)

Current/Pending Research Support

U01 AA13518-01 Adron Harris (PI) 25% effort
9/28/01 - 9/27/06
NIH/NIAAA
"INIA: Microarray Core"
This proposal was a response to the Integrative Neuroscience Initiative on Alcoholism (INIA) RFA-AA-01-002. The overall goal is to support the use of microarray technology to define changes in gene expression that either predict or accompany excessive alcohol consumption.
Role: Co-investigator

003658-0223-2001 Iyer (PI) 16% effort
01/01/02 - 08/31/04
Texas Higher Education Coordinating Board (ARP)
"Microarray based global mapping of DNA-protein interactions at promoters in human cells"
This is a pilot project to map the in vivo interactions of transcription factors with human promoters
Role: PI

Information Technology Research 0325116 R. Mooney (PI) 9% effort
09/01/03 - 08/31/07
NSF
"Feedback from Multi-Source Data Mining to Experimentation for Gene Network Discovery"
Role: Co-investigator

1 R01 CA95548-01A2 (pending) Iyer (PI) 25% effort
12/1/03 – 11/30/08
NIH
"Analysis of genome-wide transcriptional control in yeast"
This is a project to identify stress responsive transcription factor targets in yeast through the use of DNA microarrays
Role: PI

Breast Cancer Idea Award (pending) Iyer (PI) 10% effort
1/1/04 – 12/31/06
US Army Medical Research and Materiel Command
"Genome-wide chromosomal targets of oncogenic transcription factors"
This is a project aimed at identifying direct chromosomal targets of c-myc and ER in human cells through the use of a novel sequence tag analysis method.
Role: PI
003658-0531-2003 (pending) Marcotte (PI) 8% effort
01/01/04 - 12/31/05
Texas Higher Education Coordinating Board (ATP)
"Cell arrays: A novel high–throughput platform for measuring gene function on a genomic scale"
This proposal is aimed at developing a novel microarray based platform for automated, high–throughput microscopic imaging of cells, allowing rapid and systematic evaluation of gene function.